airway epithelium consists of squamous metaplasia/dysplasia/carcinoma *in situ* associated with invasive squamous cell carcinoma, which is very rare in mouse lung. Interestingly, the treatment with N-nitroso-tris-chloroethylurea by skin painting of inbred mice induced pulmonary squamous cell carcinoma with premalignant changes in a strain-dependent manner (9). Preliminary reports of GEM models include induction of the squamous differentiation program by overexpression of human keratin 14 in the airway epithelium (10), and involvement of Lkb1 with mouse squamous cell lung cancer (11).

New directions for mouse models

A newer generation of compound conditional mouse models are aimed to integrate varied features of existing models and develop new ones (1). For example, lung tumors in compound mice with conditional mutations in the K-ras proto-oncogene and different alleles of p53 tumor suppressor gene are associated with stromal metaplasia and distant metastases characteristic of human lung adenocarcinomas. It has become obvious that human lung cancer has complex genetics which will require complex mouse models.

Recent studies of cross-species comparisons of cancer signaling have detected marked similarities in mouse and human lung tumorigenesis using gene profiling (12,13). This approach has also been extended to establish signatures for aggressive tumors of lung and other organs using SV40 transgenic mice (14). The reports provide a new molecular criterion for validating lung tumor models and potential clues for yet unappreciated pathways in human tumors.

Advances in imaging technologies parallel development of GEM. Discoveries through gene expression profiling in GEMs have led novel in vivo imaging techniques of mouse lung tumors using an optical probe activated cathepsin proteases (15). Yet another approach has been the generation of a conditional luciferase reporter mouse that enables bioluminescence imaging of tumorigenesis (16).

The most frequently used model for chemoprevention studies has been the A/J mouse. Recent studies have incorporated the mutant A/J mouse with genetic alterations in p53, K-ras or Ink4A/Arf, which are commonly involved in human lung tumorigenesis and facilitate the adenoma-carcinoma assessment (17,18). Moreover genetic signatures for such chemopreventive agents as green tea and budenoside and treatment with Gefitinib have been established using cDNA microarray analysis on mouse lung tumors (19, 20).

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M10-04 Emerging Field of Lung Cancer Research, Tue, Sept 4, 10:30 - 12:00

Telomerase immortalized human bronchial epithelial cells (HBECs) have stem cell characteristics

Shay, Jerry W.; Sato, Mitsuo; Lee, Woochang; Girard, Luc; Xie, Xianjin; Xie, Yang; Sullivan, James; Spinola, Monica; Minna, John D.

University of Texas Southwestern, Dallas, TX, USA

Background.

The expression of the catalytic subunit of human telomerase (hTERT) produces telomerase activity and circumvents telomere-based replicative aging in normal cells. We have used hTERT to immortalize a variety of human cell types including skin keratinocytes and fibroblasts, muscle satellite cells, breast epithelial and stromal fibroblast cells, and both corneal epithelial cells and fibroblasts (keratocytes). Corneal epithelial cells expressing introduced hTERT require growth on collagen IV while both breast epithelial cells and skin keratinocytes require feeder layers to prevent a premature p16-induced growth arrest due to inadequate culture conditions. Human corneal and skin cells expressing hTERT can be used to form organotypic (3D) cultures. Stromal cells are mixed with collagen type I, permitted to contract, and then placed on a porous membrane in a transwell dish. The hTERT immortalized epithelial cells are then layered on top of the stromal cell/collagen plug the cells are exposed to air instead of being submerged in culture medium. Such organotypic cultures express differentiation-specific proteins, demonstrating that hTERT does not grossly alter the differentiation phenotype.

Immortalization of Human Bronchial Epithelial Cells (HBECs) with CDK4 and hTERT.

The optimal culture conditions to immortalize human bronchial epithelial cells (HBEC) with only ectopically introduced hTERT have not been determined, and thus we expressed both hTERT and cdk4 (to bypass upregulated p16). In organotypic (3D) culture, cdk4 + hTERT immortalized human bronchial epithelial cells are placed on top of collagen plugs contracted by normal human lung fibroblasts and then exposed to an air-medium interface and allowed to differentiate. One immortal clone HBEC3-KT is able to differentiate into both mucous (goblet) and ciliated epithelial cells when placed in 3D cultures. Thus these cells may have wide utility in investigating normal morphogenesis and diseases associated with lung dysfunction. In addition, these cells also provide a novel cellular resource to study the molecular pathogenesis of lung cancer.

Characterization of HBECS.

In addition to studying their ability to differentiate in 3D organotypic culture, we have performed expression profiling (Affymetrix and Illumina arrays) and Q RT-PCR expression profiling of the immortalized compared to non immortal HBECs. We have defined gene expression signatures, of a panel of 30 stem cell genes that show dramatic expression changes with immortalization (such as changes in Notch1, Hey2, Jag1, DLL1, Sox2, and Smo).

Isogenic Oncogenic Manipulation of HBECs.

In the HBEC3-KT immortalized cells we have stably knocked down p53 using a shRNA and also over-expressed the transforming oncogene, K-rasV12. The HBEC3-KT cells with knockdown of p53 and over-expression of K-rasV12 invade into the fibroblast stromal matrix

in 3D cultures instead of differentiating into mucous and ciliated differentiated cells. These cells can also grow as large colonies when placed in soft agar and when such colonies are introduced into the lungs of immunosuppressed mice, some colonies develop into squamous cell carcinoma and in other animals they develop into adenocarcinoma. This suggests that these immortalized and transformed HBECs have bronchiolar-alveolar stem-like characteristics that can differentiate into squamous cell and adenocarcinoma non small cell lung cancer.

Stem cells are defined by both their ability to make more stem cells (self renewal) and their ability to produce cells that can differentiate. Human bronchial epithelial cells expressing cdk4 and hTERT fulfill this definition of normal stem cells by continuous self renewal and by having the capability of differentiating into both mucous and ciliated epithelial cells. Thus, 3D organotypic cultures bridge standard monolayer culture (2D) with those of animal models of lung cancer and should be important for developing a fuller understanding of the pathogenesis of human lung cancer. In summary, the production of hTERT engineered cells offer the possibility of producing tissues to model a variety of diseases and aged-related medical conditions.

Targeting Telomerase in Lung Cancer Stem Cells.

Similar to normal stem cells, cancer stem cells also have the ability to self-renew as well as undergo differentiation to give rise to the phenotypically diverse types of cancer cells. The implications of this is if only a rare subset of tumor stem cells drives tumor formation, then the goal of cancer therapy should be to identify this population of cells and to develop therapies that target unique mechanisms that are only active in cancer stem cells, sparing normal tissue stem cells. As part of our anti-telomerase therapeutics program, we have addressed the following question: Will telomerase inhibitors not only target the bulk of the tumor cell population but also the rarer cancer stem cells?

Normal stem cells have longer telomeres compared to cancer stem cells, thus we believe there may be a window of opportunity to target cancer stems cells by inhibiting telomerase and driving telomeres short and cells into apoptotic cell death, hopefully without irreversible damage to normal stem cells. While the characteristics of the cancer stem of human lung cancer are not presently known, we have examined almost all cancer stem cell putative markers. In each case we have observed that purified lung cancer cells with putative markers for cancer stem cells are positive for telomerase activity and that an inhibitor currently being tested in early stage clinical trials, GRN163L, robustly inhibits the activity of telomerase in these sorted populations of cells as well as the mass population of cancer cells.

Lung Cancer Stem Cell Like Properties Are Enriched in Lung Cancer Spheroids.

We have begun to culture lung cancer cells as spheroids on non-adherent culture dishes and have observed that many stem cell markers (e.g. Oct 4, Nanog, SOX2) are highly up-regulated when cultured as spheroids as opposed to normal adherent cell cultures. When such human lung spheroids are treated with GRN163L, we observe a decrease expression of these stem cell markers. Thus we propose that while cancer stem cells are maintained at low numbers in most solid tumors, the treatment of lung tumors with GRN163L may shift the stem cell population into a depletion mode eventually leading to loss of the putative stem cell population. In summary, telomerase is a universal oncology target with high tumor specificity and we propose that anti-telomerase therapies (such as GRN163L) may target the cancer stem cell population as well as the bulk of the tumor.

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Session M11: Chemoprevention

M11-01 **Chemoprevention, Tue, Sept 4, 10:30 - 12:00**

Molecular analysis of preinvasive lesions in search for new surrogate biomarkers of chemopreventive drug efficacy.

Massion, Pierre P.

Vanderbilt University, Nashville, TN, USA

To address the lung cancer epidemic, beside antitobacco primary prevention efforts, early detection and chemoprevention strategies are needed. A better understanding of lung carcinogenesis is critical to improve chemoprevention strategies. The study of molecular abnormalities in preinvasive lesions represent one way to address the critical steps of lung tumor development. The identification of molecular events involved in preinvasive lesions progression could lead to clinical studies with new molecular-targeted agents and the development of surrogate biomarkers. Such biomarkers are essential to select high-risk patients, select chemoprevention strategies and monitor drug efficacy.

While morphological features of the airway mucosa of high risk individuals are well established, by themselves do not provide a satisfying predictive model of lung cancer development. The hypothesis pursued here is that molecular characteristics of the airway mucosa contains such information and that when applied with epidemiological, morphological and imaging related data elements, would provide a powerful tool to classify subjects at high risk for lung cancer and should ultimately personalize the management of lung cancer and reduce the overall mortality associated with this disease.

Access to these preinvasive lesions is provided by selection of highest risk group, autofluoresence bronchoscopy or other endoscopic techniques that improve the sensitivity of detection of preinvasive dysplastic bronchial lesions. Morphological features and molecular abnormalities of such lesions have been described and can be monitored by follow-up bronchoscopies to validate potential chemoprevention treatments.

Bronchial specimen obtained from these high risk individuals lead to the report of a series of candidate biomarkers. These markers are being discovered through a series of methodologies advances that include polymerase chain reaction, genome-wide DNA copy number alterations, expression arrays and proteomic strategies such as conventional